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Short communication

Evolution of relative lower molar sizes among local populations of the raccoon dog (*Nyctereutes procyonoides*) in Japan

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Inter-specific variations in relative molar sizes (size-proportion of the first, second, and third lower molars) in murine rodents, canids, and other mammals reflects their dietary habits (Kavanagh et al. 2007; Asahara 2013; Halliday and Goswami 2013). These studies have suggested that carnivorous species tend to have larger lower first molars (M_1) and smaller distal molars, and omnivorous species tend to have smaller M_1 and larger distal molars. This is especially true in Canidae, where inter-specific variation is unique and relative M_1 size can vary in relation to other mammals, which may have contributed to the large diversity in dietary habits among canids (Asahara 2013). This difference evolved in parallel in many lineages of canids, and relative molar sizes may evolve easily (Asahara 2013). Previous studies have focused on inter-specific variation; however, the level of inter-population variation has not been previously investigated. Therefore, the variability, evolvability (ability to respond selection: Houle 1992), and evolutionary rapidity of relative molar sizes whether the trait evolve at the inter-population level or not are still not clear, while this trait seems to evolve easily that single inhibition/activation dynamics during teeth development can generate its variation (Kavanagh et al. 2007).

Japanese raccoon dogs (*Nyctereutes procyonoides*) were considered to have separated into two subspecies, i.e., *N. p. viverrinus* distributed south of the Tsugaru Strait and *N. p. albus* distributed in Hokkaido (Saeki 2009). Whereas the raccoon dog is an omnivorous animal eating various foods including fruits, invertebrates, and small vertebrates (Saeki 2009), several studies have reported differences in cranial morphology between the two Japanese subspecies (Yoshiyuki 1988; Haba et al. 2008; Asahara 2014), and suggested that dietary habits of the Hokkaido subspecies *N. p. albus* are more carnivorous than those of the southern subspecies *N. p. viverrinus* (Haba et al. 2008). However, among raccoon dog populations, relative molar sizes, which reflect the diet at an inter-specific level were not compared. In this study, I investigated relative molar sizes among raccoon dog populations in Japan to clarify the evolution and evolvability of this trait at the inter-population level.

Materials and methods

I examined 273 raccoon dog (*Nyctereutes procyonoides*) mandible specimens from six locations in Japan (Hokkaido, 49 specimens; Niigata, 9; Gifu, 153; Chiburi, 36; Shimane, 21; Miyazaki, 5; Fig. 1; Appendix 1). Among these specimens, 102 were males, 100 were females and the other 71 were of unknown sex.

I measured molar sizes in the lower dentition. Molar sizes were measured as the projected area in photos taken from the occlusal view (Fig. 2a) using ImageJ software (NIH, Bethesda, MD). I compared the relative molar sizes in the morphospace: M_2 size/M_1 size versus M_3 size/M_1 size (abbreviated as M_2/M_1 vs. M_3/M_1; Kavanagh et al. 2007). Differences in M_2/M_1 scores among populations were tested by U-test with post hoc Holm-Bonferroni

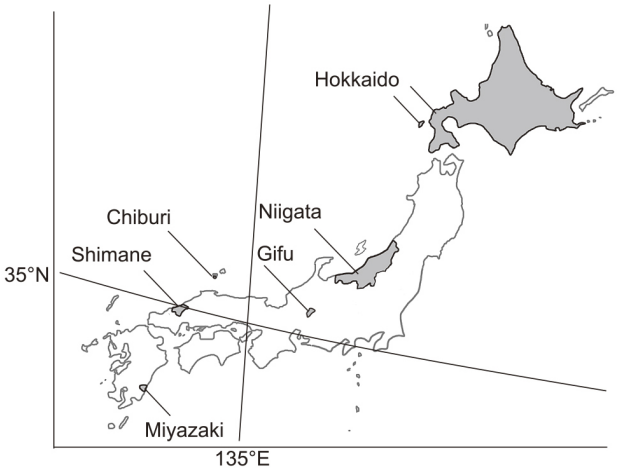

Fig. 1. Locations of the six raccoon dog (*Nyctereutes procyonoides*) populations studied (modified from Asahara 2014).

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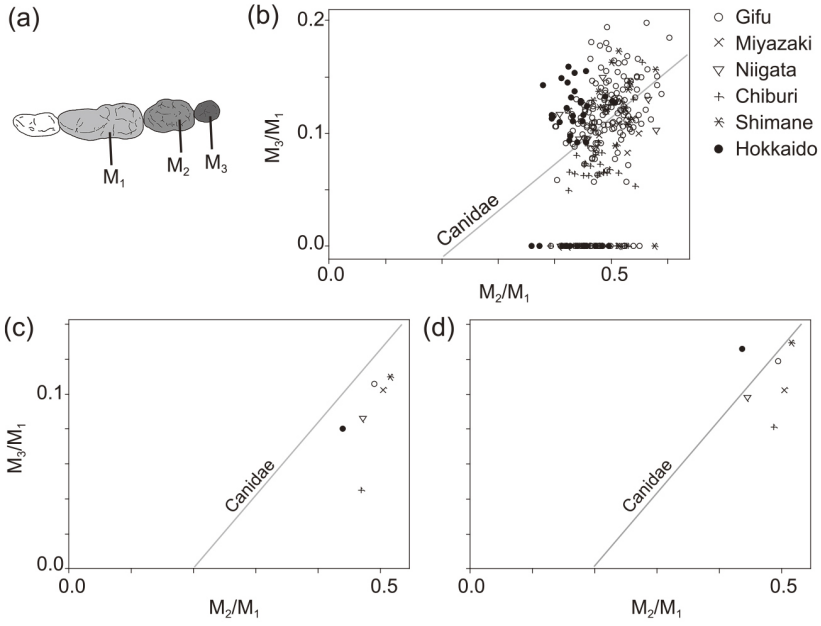

Fig. 2. Method of measuring teeth and bivariate plots of M_2/M_1 score against M_3/M_1 score. (a) Occlusal view of the lower dentition from P_4 to M_3. Colored areas are the measure of the molar size. Bivariate plots (b) by individual, (c) by population-average including congenital M_3 loss, and (d) by population-average excluding congenital M_3 loss. The grey line indicates interspecific regression in Canidae (Asahara 2013).

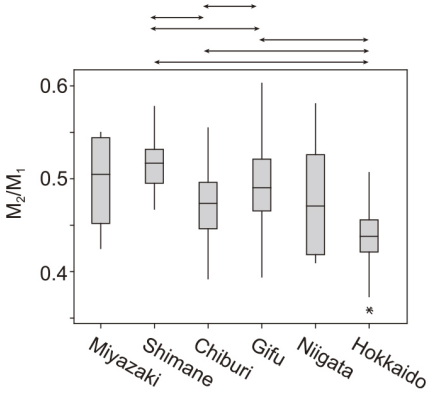

Fig. 3. Box plots of the M_2/M_1 scores for each raccoon dog population. Boxes indicate quartiles, central-lateral bars indicate averages, vertical bars indicate the range of the specimens, and asterisks indicate outliers. Significantly different pairs are indicated by arrow-lines.

correction. Within populations, sexual dimorphism was not detected (U-test); therefore, males and females were pooled. In this comparison, I did not omit specimens in which M_3 had been congenitally lost (congenital loss is defined as the absence of a tooth without any evidence of concrescence). This is because missing of M_3 is caused by an inhibitory cascade, i.e., the developmental mechanism which governs relative molar sizes (Kavanagh et al. 2007; Asahara 2013) and exclusion of specimens with dental anomalies would cause the underestimation of relative molar sizes. However, no individuals had lost M_1 or M_2, and I used M_2/M_1 as the reliable index of relative molar sizes. Statistical analyses were performed using Minitab 14 (Minitab, Inc., PA).

Results and discussion

Most individual plots were located along the regression line of interspecific variation in canids reported by Asahara (2013), while several individuals were considered to exhibit a congenital loss of M_3 and deviated from the regression line (Fig. 2b). This deviation may be caused by the fact that teeth germ smaller than some threshold tend to disappear during development (Gruneberg 1951; Wolsan 1989; Szuma 2003), as discussed by Asahara (2013). Averages of the populations were plotted below the regression line. This was especially true in the Chiburi population, which had the greatest deviation (Fig. 2c). The Chiburi population tended to lose their M_3 (56% of individuals exhibit M_3 loss; Asahara 2013), a percentage which is larger than the other populations studied. However, when I compared averages of individuals that possessed M_3, members of the Chiburi population were still plotted below the regression line (Fig. 2d). Therefore, raccoon dogs of the Chiburi population have relatively small M_3, in addition to high rates of occurrence of M_3 loss (Fig. 2d). This peculiarity may be related to the Chiburi population being an island population introduced

Table 1. Molar ratios in the populations examined

Population	$M_2/M_1 \pm SD$	$M_3/M_1 \pm SD$
Hokkaido	0.43 ± 0.03	0.07 ± 0.06
Niigata	0.47 ± 0.06	0.08 ± 0.05
Gifu	0.49 ± 0.04	0.10 ± 0.04
Chiburi	0.47 ± 0.03	0.04 ± 0.04
Shimane	0.51 ± 0.02	0.11 ± 0.05
Miyazaki	0.50 ± 0.05	0.10 ± 0.01

from main-land of Japan (Saeki 2009); it may be caused by the founder effect. It further suggests that genetic drift and the founder effect can be important causes of dental evolution, as discussed in the Japanese mole *Mogera* (Asahara et al. 2012).

Population averages of the relative molar sizes varied slightly among populations (Fig. 2c; Table 1). M_2/M_1 scores, i.e., relative molar sizes, differed significantly among all populations with sample sizes larger than 10, i.e., among the Hokkaido, Gifu, Chiburi, and Shimane populations (Fig. 3). This result indicates that relative molar sizes evolve and differentiate easily and rapidly, while there were overlaps among populations. Other morphological traits, such as cranial tilting between the snout and neurocranium which must be an easily evolvable trait in cranial morphology that was differentiated between several raccoon dog populations (Asahara 2014) may be less evolvable than the relative molar sizes; the PC2 score as the index of cranial tilting is not different between the Gifu and Shimane populations (Asahara 2014), but the M_2/M_1 scores as the index of relative molar sizes differ between these populations (Fig. 3). Phylogeographic studies have reported that the Hokkaido population is nested in the genetic diversity of the Honshu and Shikoku populations and the Japanese population is not clearly differentiated (Kurose et al. 2010, 2012; Kim et al. 2013). Relative molar sizes may evolve faster than genes which are used for these phylogeographic studies e.g., cytochrome *b* and might be maintained by local selection.

In the M_2/M_1 vs. M_3/M_1 morphospace, all naturally-distributed populations were plotted along the canid inter-specific regression line reported by Asahara (2013; Fig. 2c). This result suggested that the inter-population and inter-specific variations in relative molar sizes are related to each other and may be guided by a developmental mechanism, i.e., the unique inhibitory cascade of canids (Asahara 2013).

Asahara (2013) reported that carnivorous species tend to have relatively larger M_1; they were plotted in the left lower area in the M_2/M_1 vs. M_3/M_1 morphospace. In my result, the plot of the Hokkaido population showed in the left lower area than did the other naturally distributed populations. In fact, the M_2/M_1 score of the Hokkaido population is significantly smaller than any of the other populations with sample size larger than 10 (Fig. 3). The Hokkaido population has been previously reported to exhibit morphological features that seem to reflect more carnivorous adaptations (Yoshiyuki 1988; Haba et al. 2008; Asahara 2014). The result of the present study is consistent with these previous studies. However, it is not clear whether difference in the M_2/M_1 scores between Gifu, Chiburi, and Shimane populations relates to diet because a local difference in diet is not clear among these locations.

Here, my result indicated that relative molar sizes have high evolvablility, can evolve at the inter-population level, and may reflect dietary habits. Relative molar sizes have been shown to evolve reflecting dietary adaptation in many mammalian species (Kavanagh et al. 2007; Asahara 2013; Halliday and Goswami 2013) and also correlate with the hypsodonty index that relates hervivorous adaptation in ungulates (Wilson et al. 2012). If high evolvability of relative molar sizes is shared among all mammals, it may have contributed to the parallel evolutions of this trait, which reflect dietary habits in many mammals. Further studies would provide a better understanding of the evolution of relative molar sizes and its relation to dietary adaptation at lower and higher taxonomic levels, connecting the microevolution and macroevolution of the phenotypic trait.

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Appendix 1.

Specimens examined

Locality	Specimen number	Institute
Hokkaido	9905, 11430M, 11431F, 11433, 11435, 11436, 11440, 11441, 11443, 11445, 11447, 11448, 11454, 11455, 11459, 11461, 11464, 11465, 11466, 11467, 11468, 11471M, 11472, 11473, 11474, 11475, 11477, 11478, 11479, 11480, 11482, 11483, 11484, 11485, 24940, 47615, 47616, 47617, 47618, 47620, 47623, 47624, 47625	Museum of Botanic Garden, Hokkaido University (n = 43)
	27280M, 27324M, 27325F, 27329M, 27362M, 27363F	National Museum of Nature and Science (n = 6)
Niigata	1876F, 12190F, 12192M, 14650M, 16800F, 17628, 19499, 25880, 31488	National Museum of Nature and Science (n = 9)
Gifu	245, 246M, 247M, 248M, 250F, 251M, 252M, 253F, 254F, 255F, 256F, 257F, 258F, 259M, 260M, 261F, 262F, 263M, 264M, 265M, 266F, 267M, 268M, 270M, 271M, 272F, 273F, 274M, 276F, 278M, 280M, 282F, 283M, 285M, 286F, 287F, 288F, 289F, 290F, 293M, 294M, 295M, 291F, 292M, 296M, 297M, 299M, 300M, 301F, 303F, 304F, 306F, 307M, 308M, 309M, 310F, 311F, 312M, 313F, 314F, 315M, 317F, 318F, 319F, 320M, 321M, 322F, 323F, 324M, 325F, 326M, 327M, 328F, 329F, 330F, 331F, 332M, 333F, 334M, 335M, 336M, 337F, 338F, 339F, 340F, 341M, 342F, 343F, 344F, 345, 346M, 348M, 349M, 350F, 351F, 352M, 353F, 354M, 356M, 357M, 358F, 359F, 360M, 361F, 362M, 363M, 364M, 365F, 366F, 367M, 368F, 369F, 370M, 371M, 372F, 373F, 374M, 375F, 377M, 378M, 379F, 380F, 977M, 978M, 979M, 980F, 982F 983, 989, 990, 1010M, 1011F, 1012M, 1013F, 1014F, 768, 238F, 239F, 240F, 228, 164M, 165M, 131M 132M, 90F, 82M, 84M, 59F, 74F, 77M, 39F 40M, 6M	Primate Research Institute, Kyoto University (n = 153)
Chiburi	17, 39, 10M, 13M, 14M, 15M, 16F, 18F, 21F, 22M, 24M, 28M, 29M, 31M, 32M, 34M, 37M, 38M, 40M, 41F, 46F, 49M, 50F, 53F, 55F, 57F, 59M, 60F, 6M, 74-23F, 74-44F, 74-4F, 74-51F, 75-36, 7F, 8F	The Kyoto University Museum, Kyoto University (n = 36); data were from Asahara (2013); Specimen numbers are private number of T. Ito who donated specimens to the museum
Shimane	75-1, 75-2M, 75-5, 75-6M, 75-7, 75-8, 75-13, 75-14, 75-16F, 75-17F, 75-18, 75-22, 75-23, 75-27, 75-28 75-30, 75-31, 75-32, 75-33, 75-34, 75-35	The Kyoto University Museum, Kyoto University (n = 21); Specimen numbers are private number of T. Ito who donated specimens to the museum
Miyazaki	25738M, 27351M, 28176F, 28177F, 28185F	National Museum of Nature and Science (n = 5)

M: male; F: female.